

Haugland and Yue
Serial No. 09/557,275

REMARKS

The Claimed Invention

The claimed invention is directed to unsymmetrical cyanine dyes incorporating an azabenzazolium moiety. These compounds form fluorescent complexes with nucleic acids and poly(amino acids) and are used to effectively stain such structures.

The Pending Claims

Prior to entry of the attached amendments, Claims 1-47 are pending. Claims 1-14 are directed to unsymmetrical cyanine dyes of the invention. Claims 15-23 are directed to a fluorescent complex comprising a nucleic acid polymer and the cyanine dyes of Claims 1-14. Claims 24-27 are directed to complex comprising a poly(amino acid) and cyanine dyes of Claims 1-14. Claims 28-38 are directed to methods for staining poly(amino acids). Claims 39-43 are directed to methods for staining nucleic acids. Claims 44-47 are directed to kits comprising cyanine dye compounds of Claims 1-14.

The Office Action

Claims 1-47 are restricted.

Amendments

Claims 1-16, 18-24, 26-27, 44 and 46-47 have been amended.

Claims 1, 15, 24, 28, 39 and 44 have been amended to correct a typographical error in the structure as drawn. Support can be found in original Claim 2 as filed and on 6 line 27; p. 57 lines 26-31 to p. 58 lines 1-24; p. 66 compound 14 and 15; p. 67 compound 16 and 17; p. 68 compound 18-20; p. 69 compound 21, 44 and 22; p. 70 compound 23; p. 71 compound 24 and 25; p. 72 compound 26 and 27; p. 73 compound 47 and 28; p. 74 compound 45 and 46; p. 75 compound 29 and 30; p. 76 compound 41 and 31; p. 77 compound 32 and 33; p. 78 compounds 34-37; p. 79 compounds 38-40 and 42; p. 80 compound 43 and 48; p. 81 compound 49 and 50; p. 82 compound 51 and 52; p. 83 compound 83 and 54; p. 84 compound 55; p. 85 compound 56 and 57; p. 86 compound 58 and 59; p. 87 compound 60.

Haugland and Yue
Serial No. 09/557,275

Claims 2-14, 16, 18-22, 25-27, and 46-47 have been amended to replace 'A' with 'The' to indicate dependent form.

Claims 1-3, 5-6, 11-12, 14-15, 22, 24, 44 and 47 have been amended to clarify the Markush groups.

Claims 1, 15, 19, 24 and 44 have been amended to accurately define the A moiety of the claimed compounds. Support can be found in original filed claim 2 and page 4 lines 14-20.

Claims 1, 15, 19, 24 and 44 have been amended include the substituents hydrogen and methylthio of the aromatic carbon atoms of the A moiety. Support can be found page 5 lines 16-22; page 65 compound 11 and page 78 compound 35.

Claims 1, 15, 19, 24 and 44 have been amended to include the definition of β . Support can be found on page 5 lines 5-14.

Claims 1, 15, 19, 24 and 44 have been amended to replace the R2 substituent 'sulfonate' with 'sulfo'. Support can be found on page 5 line 26.

Claims 1, 15, 19, 24 and 44 have been amended to replace '-(CH₂)₂-V-(CH₂)₂-' with '-(CH₂)₂-W-(CH₂)₂-'.

Claims 1, 15, 19, 24 and 44 have been amended to accurately define the ring structure that can be formed by R⁶ and R⁷. Support can be found on page 9 lines 14-26; page 10 lines 1-10 and original filed claim 2.

Claims 1, 7-9, 11, 14-15, 19, 24 and 44 have been amended to replace BRIDGE-DYE with BRIDGE. The scope of the claims has now been limited to monomers of a dye or dimmers comprising DYE. Support can be found in the original filed claims and on page 18 lines 20-23; page 19 lines 1-7 and lines 30-31; page 20 lines 1-2.

Claim 3 has been amended to accurately define the substituents of alkyl substituted R²¹, R²² or R²³. Support can be found on page 13 lines 30-31; page 14 lines 1-4.

Claim 5 has been amended to accurately define CYCLIC SUBSTITUENT. Support can be found on page 11 lines 31; page 12 lines 1-7.

Claim 6 has been amended to accurately define TAIL. Support can be found in original filed claim 3 and page 12 lines 11-22 and page 13 lines 20-22.

Claim 12 has been amended to include 'a DNA-binding protein'. Support can be found in original filed claim 13 and page 24 line 30.

Haugland and Yue
Serial No. 09/557,275

Claim 15 has been amended to clarify that that fluorescent complex comprises a) a compound and b) a nucleic acid polymer. Support can be found in the original filed claim and page 3 lines 20-22; page 39 lines 7-24; page 87 Example 32; page 89 Example 33; page 90 Example 34; page 91 Example 35; page 92 Example 36; and page 94 Example 37.

Claim 16 has been amended to include the imitation of nucleic acid polymer fragments. Support can be found on page 32 lines 8-12.

Claim 17 is ~~cancelled~~ without prejudice.

Claim 18 has been amended to clarify the samples comprising nucleic acid polymers. Support can be found on page 32 lines 23-26 and page 39 lines 30-31.

Claim 19 has been amended to clarify the medium that the fluorescent nucleic acid complex can be present in. Support can be found on page 39 lines 21-23.

Claim 21 has been amended to include a cell as a biological structure. Support can be found in original filed claim 20 and page 32 lines 23-28.

Claim 23 is ~~cancelled~~ without prejudice.

Claim 24 has been amended to clarify that the claimed composition is a complex of the aza cyanine dye and a poly (amino acid). Support can be found in the original filed claim and page 3 lines 22-23; page 42 lines 4-6; page 94 Example 38; page 95 Example 39 and Example 40.

Claims 28-43 have been provisionally withdrawn.

Claim 44 has been amended to clarify that the claimed kit contains a stock solution and a buffer for dilution of the stock solution and that the stock solution comprises one or more compounds. Support can be found in original filed Claim 44 and 45; page 51 lines 21-30 and page 52 lines 4-6.

Claim 45 is ~~cancelled~~ without prejudice.

Support for new Claims 48-74 is found in the claims as filed and throughout the specification, and in particular the limitations associated with the compounds of claims 52-74 can be found in compound 14 (page 66); compound 15 (page 66); compound 16 (page 67); compound 44 (page 69); compound 25 (page 71); compound 27 (page 72); compound 47 (page 73); compound 28 (page 73); compound 46 (page 74); compound 35 (page 78); and compound

Haugland and Yue
Serial No. 09/557,275

40 (page 79). Additional support for the staining solution of Claims 69-74 can be found on pages 29-31. Support for new claim 48 can be found in original filed claim 44 and page 19 lines 30-31. Support for new claims 49 and 50 can be found on page 51 lines 21-25.

Applicants believe that no new matter has been added by any of these amendments and the Examiner is respectfully requested to enter them.

RESPONSE TO THE RESTRICTION REQUIREMENT

In the response that follows, the Examiner's Election/Restriction of the Applicant's claimed invention is provided in full text, as identified by indented small bold print, followed by the Applicants response.

35 U.S.C. 121 Restriction

- I. **Claims 1-27 and 44-47 drawn to azo dye compositions, classified in class 532, subclass 573 for example.**
- II. **Claims 28-43, drawn to a method of staining nucleic acids, classified in class 436, subclass 94 for example.**

Applicants respectfully traverse the above restriction requirement and request reconsideration. As required by CFR 1.143, Applicants provisionally elect Group I as drawn to the azo dye compounds.

The invention of group I, and the invention of group II are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product claimed can be used in a materially different process of using the product (MPEP § 806.05(h)). In the instant case nucleic acids may be tagged or labeled with fluorescent dyes other than azo cyanine dyes, for example, they may be labeled with rhodamine or fluorescein. Further nucleic acids may also be tagged or labeled with oligonucleotide fluorescent intercalating agents or with lanthanide elements for detection with or without the invention of the azo cyanine dyes of group I.

Applicants respectfully submit while additional compounds other than compounds of the present invention may be used to 'tag or label' nucleic acids, the compounds of the present

Haugland and Yue
Serial No. 09/557,275

invention possess unique properties that exclude the use of the Examiner's cited compounds for such methods. Among these unique properties include: (1) increased fluorescence upon formation of a complex, (2) cell permeability properties, (3) wavelength emission spectra and (4) formation of dimmers that permits the staining of an analyte of interest such as nucleic acid polymers in the instant methods.

First, the compounds of the present invention are virtually non-fluorescent when in solution and become fluorescent only after forming a complex with an analyte of interest. When used in methods to stain an analyte of interest in a blot or gel, this unique property overcomes the need for a washing step, providing a method that requires less time than traditional stains that require stringent washing steps to remove unbound fluorescent compounds. In addition, this increased fluorescence property allows for staining of nucleic acids in solution or in another medium wherein the nucleic acid is not attached to a solid or semi-solid surface. This is an application not possible with the compounds cited by the Examiner.

Second, the compounds of the present invention are cell-permeant, especially when present as monomers. Such cell permeability, in conjunction with the ability to increase fluorescence when present as a complex, permits the compounds of the present invention to be used in live cells. Staining an analyte of interest in a live cell offers many applications for the compounds of the present invention such as functioning as tracers or viability markers. The properties of the instant compounds also importantly allows for these compounds to be used in functional assays where removal of unbound compounds is not feasible. The compounds cited by the Examiner cannot be used for such methods.

Third, the compounds of the present invention possess unique wavelength emission spectra not observed by the compounds cited by the Examiner. This unique property is facilitated by the aza-benzazolum moiety and permits the compounds of the present invention to be used in methods that would not be possible with the compounds cited by the Examiner. For example, the compounds of the present invention can be combined with dyes of different wavelengths for use in multiple dye staining techniques whereby the emission from different dyes can easily be distinguished from each other. As the compounds of the present invention possess unique wavelength spectra, they effectively increase the multiplexing applications that

Haugland and Yue
Serial No. 09/557,275

can be performed. The compounds cited by the Examiner have different wavelengths than the compounds of the present invention and are not typically used in multiplexing applications.

Fourth, the compounds of the present invention can be used in the instant methods when present as dimers. The dimers possess a unique property of a more stable complex with an analyte of interest than the monomers of the present invention. This is an important advantage because it allows for preformed complexes of the compounds and an analyte of interest. This unique property serves two purposes: (1) it allows for a quick determination of the relative amount of analyte of interest and (2) it allows further analysis due to the stable complex formation wherein the complex could be analyzed by gel electrophoresis. For example, a sample thought to contain an analyte of interest could be mixed with a dimer of the present invention that would (1) indicate whether the analyte of interest was present due to the enhanced fluorescence when a complex is formed and if present in a satisfactory amount (2) and allow further analysis on a gel. Thus, the dimers used, in combination with the unique property of the compounds of the present invention of being virtually non-fluorescent when in solution allows for methods not possible with the compounds cited by the Examiner.

Thus, the compounds of the present invention possess many unique properties that are not present in the compounds cited by the Examiner. Moreover, the compounds cited by the Examiner are not feasible compounds for the present methods. Therefore, due to the unique properties of the present compounds, as described above, Applicants respectfully request that the Examiner reconsider the restriction requirement and rejoin the claims of Group I with Group II.

This application contains claims directed to the following patentably distinct species of the claimed invention: α , β , A, ψ , L, R₁-R₂, R_x, Sc, V, X, Y, Y_m, Y_p, m, n, p, CAP, CAP', TAIL, BRIDGE, BRIDGE-DYE, CYCLIC SUBSTITUENT, SPACER, SPACER' SPACER-CAP, AND LINK-SPACER-CAP.

Applicant is required under 35 U.S.C. 121 to elect a single disclosed species for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, claims 1-47 are generic.

Applicant is advised that a reply to this requirement must include an identification of the species that is elected consonant with this requirement, and a listing of all claims readable thereon, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

Haugland and Yue
Serial No. 09/557,275

Applicants provisionally elect the species compound comprising A. The Applicants would like to point out that the point of novelty for the present invention is the A moiety. Thus, when the other species are combined with the A moiety the compounds are patentably distinct from other compounds that are not within the scope of the present invention. However, the species identified above are not patenably distinct from each other.

Claim 1-47 and new claims 48-74 either directly contain the A species as elected above or depend from a claim that contains the A species.

CONCLUSION

In view of the above amendments and remarks, it is submitted that this application is now ready for allowance. Early notice to this effect is solicited. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (541) 984-5656.

Respectfully submitted,

Date: Aug. 21, 2002

Koren J. Anderson
Koren J. Anderson, PhD
Reg. No. 51,061

Molecular Probes, Inc.
P.O. Box 22010
Eugene, Oregon, 97402
Phone: (541) 984-5656
Facsimile: (541) 465- 8354